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5-Aminolevulinic acid and its derivatives: physical chemical properties and protoporphyrin IX formation in cultured cells

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Abstract

Protoporphyrin IX (PpIX) is used as a fluorescence marker and photosensitizing agent in photodynamic therapy (PDT). A temporary increase of PpIX in tissues can be obtained by administration of 5-aminolevulinic acid (ALA). Lipophilicity is one of the key parameters defining the bioavailability of a topically applied drug. In the present work, octanol–water partition coefficients of ALA and several of its esters have been determined to obtain a parameter related to their lipophilicity. The influence of parameters such as lipophilicity, concentration, time, and pH value on PpIX formation induced by ALA and its esters is then investigated in human cell lines originating from the lung and bladder. ALA esters are found to be more lipophilic than the free acid. The optimal concentration (c_{opt}, precursor concentration at which maximal PpIX accumulation is observed) is then measured for each precursor. Long-chained ALA esters are found to decrease the c_{opt} value by up to two orders of magnitude as compared to ALA. The reduction of PpIX formation observed at higher concentrations than c_{opt} is correlated to reduced cell viability as determined by measuring the mitochondrial activity. Under optimal conditions, the PpIX formation rate induced by the longer-chained esters is higher than that of ALA or the shorter-chained esters. A biphasic pH dependence on PpIX generation is observed for ALA and its derivatives. Maximal PpIX formation is measured under physiological conditions (pH 7.0–7.6), indicating that further enhancement of intracellular PpIX content may be achieved by adjusting the pharmaceutical formulation of ALA or its derivatives to these pH levels.

Keywords: 5-Aminolevulinic acid; 5-Aminolevulinic acid esters; Photodynamic therapy (PDT); Fluorescence; Protoporphyrin IX; ALA esters; Lipophilicity

1. Introduction

The exogenously stimulated formation of intracellularly generated protoporphyrin IX (PpIX), a precursor of heme, is becoming one of the fastest developing areas in the field of photodynamic therapy (PDT) and fluorescence photodetection (PD) of malignant and non-malignant diseases (see Ref. [1] and references therein). In most clinical and preclinical studies, systemic or topical application of 5-aminolevulinic acid (ALA) is used to temporarily increase the concentration of PpIX in the target tissues. Administration of ALA, a metabolic precursor in the biosynthetic pathway of heme, bypasses the negative feedback control exerted by heme on the enzymatic step in ALA synthesis. Although PpIX formation is present in nearly every nucleated cell, preferential formation and accumulation of this photosensitizer have been demonstrated in tissues known to have a high cellular turnover. The main reason for a somewhat selective PpIX accumulation in the latter cell types is still not completely understood. Experimental evidence has been found that, in some tumors, the ferrochelatase activity is reduced, while the activity of the porphobilinogen deaminase is enhanced [2,3]. For historical reasons [4] and due to the ease of administration to the skin of both drug and light, the main applications of ALA-mediated PpIX therapy are in dermatology. This modality is now in Phase III trials for the treatment of actinic keratosis and has also been employed clinically for the treatment of basal cell carcinoma. Recently, other medical fields, namely pulmonology [5], urology [6–9], gastroenterology [10], ENT [11], gynecology [12], and neurosurgery [13], have implemented this technique for the improved management of cancer. In addition to its tumor selectivity, the administration of ALA prevents prolonged cutaneous photosensitivity, one of the major drawbacks of some of the earlier photosensitizers [14].

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Despite promising results, it appears that this methodology is open to quite significant improvement, in particular in the case f topically applied ALA. Since ALA is a hydrophilic molecule, its penetration through cellular membranes and into the interstitial space of tissues is low. Hence, ALA-induced PpIX formation is ften limited to superficial tissue layers. Furthermore, PpIX formation shows considerable heterogeneity when ALA is applied topically. Both inhomogeneous and limited tissue distribution result in nonefficient treatment of deeper-lying or nodular lesions, even if light in the red region of the PpIX absorption spectrum is used [15,16]. Since deeper-lying lesions are often not accessible by PDT, they are missed after topical application of ALA. Consequently, relatively high doses of ALA have to be applied over long periods of time, increasing the risk of complications [17,18].

Due to these drawbacks, PpIX-mediated PDT and diagnosis have recently been started with more lipophilic derivatives of ALA in order to enhance the poor bioavailability of ALA. Several groups have shown that using such ALA prodrugs may enhance the PpIX concentration by up to two orders of magnitude as compared with the parent molecule [19–23].

Since lipophilicity is one of the key parameters, in the present study the octanol–water partition coefficient P of some alkyl esters has been determined as a measure related to this property. With the final goal of defining clinical protocols with improved bioavailability of ALA, we investigated the impact of lipophilicity, pH value, concentration and duration of exposure of ALA and its derivatives on PpIX formation and cell viability. This was performed by means of fluorescence spectroscopy of PpIX using four different human cell lines. It was demonstrated that long-chained ALA derivatives and physiological pH values resulted in the highest relative fluorescence values. Using ALA derivatives, the choice of the optimal concentration of the PpIX precursor was shown to be of major importance for cell viability and maximal PpIX formation.

2. Materials and methods

2.1. Chemicals

ALA hydrochloride, ALA-methylester hydrochloride (m-ALA), (3-4,5-dimethylthiazol-yl)-2,5-diphenyl tetrazolium bromide (MTT), and *n*-octanol were purchased from Sigma (Fluka, Buchs, Switzerland). 0.1 N NaOH was obtained from Merck (Darmstadt, Germany). Other ALA esters (Table 1) were synthesized in our laboratories following the procedure described recently by Kloek et al. [20].

2.2. Determination of physicochemical properties

The apparent partition coefficients (P) of ALA and its esters were determined in an octanol–buffer system at 21°C.

Table 1
List of hydrochlorides of ALA and esters used for in vitro experiments ($HCl \cdot R^2N–CH_2–CO–CH_2–CH_2CO–OR^1$ = general structure)

Compound	R^1	R^2	Mol. mass [g/mol]	Abbreviation
ALA	H	H	167.6	ALA
ALA-methylester	CH_3	H	181.6	m-ALA
ALA-ethylester	CH_2CH_3	H	195.6	e-ALA
ALA-butylester	$(CH_2)_3CH_3$	H	223.8	b-ALA
ALA-hexylester	$(CH_2)_5CH_3$	H	251.8	h-ALA
ALA-octylester	$(CH_2)_7CH_3$	H	279.6	o-ALA
ALA-cyclohexylester	C_6H_{11}	H	249.8	ch-ALA

The aqueous phase was a 0.1 M phosphate buffer (PBS) solution of pH 7.4. The PBS solution and the octanol were mutually saturated before use by shaking 300 ml of PBS with an equal quantity of octanol for 30 min. Twenty milligrams of the compound to be investigated were dissolved in 10 ml of the aqueous phase and an equal quantity of octanol was added. The mixtures were shaken for about 30 min and left for phase separation overnight at 4°C. Absorption of both phases was measured with a UV–Vis absorption spectrometer (Cary 5, Varian, Australia) at 269 nm (see Fig. 1(a)). The partition coefficients P were calculated according to:

$$P = c_{oct}/c_{PBS} = abs_{oct}/abs_{PBS}$$

where c_{oct} and c_{PBS} represent the solute concentrations in the organic and the aqueous phase, respectively, abs_{oct} the absorption of the compound measured in the octanol and abs_{PBS} the absorption in the PBS solution (see Fig. 1(b)). The use of low concentrations and storage at low temperatures impaired the formation of dimerization products. The absence of these products was confirmed by the absence of characteristic absorption bands in the absorption spectrum of the measured solutions.

Values of acidity constants of ALA and its esters were measured by means of potentiometric titration with a standard

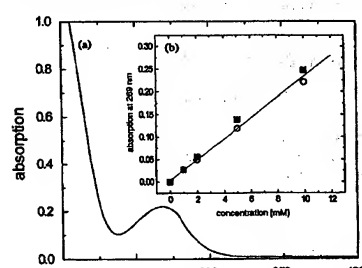

Fig. 1. (a) Absorption spectrum of b-ALA (10 mM) in PBS. (b) Absorption at 269 nm as a function of b-ALA concentration, (■) in PBS, (○) in octanol.

pH electrode (Bioblock, Frenkendorf, Switzerland). In brief, 20 mg of the corresponding drug were dissolved in 10 ml of demineralized water and titrated with 0.1 N NaOH solution. The pH of the solution was plotted against the total volume added.

2.3. PpIX fluorescence measurements

For fluorescence measurements, cells, subcultured in 48-well dishes, were exposed to various concentrations of the corresponding PpIX precursor and transferred into a thermostated fluorescence multiwell plate reader (CytoFluor Series 4000, PerSeptive Biosystems, Framingham, MA, USA, excitation wavelength $\lambda = 409 \pm 10$ nm, detection wavelength $\lambda = 640 \pm 20$ nm). Correction for cell autofluorescence and other offset parameters was provided by five wells not exposed to the PpIX precursors. Reference was provided by 200 μl of a Rhodamine 6G (0.1 g/l) (Lambda Physik, Göttingen, Germany) solution always present in one of the dishes.

2.4. Cell cultivation

All cell lines were from ATCC (Rockville, MD, USA) and grown as described. J82 and T24 cells were derived from human transitional cell carcinoma of the bladder, A549 cells from human lung carcinoma, and BEAS-2B cells were immortalized from normal human bronchial epithelium. Culture was performed in the presence in 10% fetal calf serum (FCS) and penicillin–streptomycin at 37°C and 6% CO_2 in a humid environment. For measurement purposes, the cells were subcultured in 48-well dishes (Costar 3548, Integra Biosiences, Cambridge, MA, USA) to give 10^5 cells/well 72 h prior to incubation with the ALA or one of its derivatives.

2.5. Determination of cell viability

The cell viability was tested by means of an MTT assay. This technique allows quantification of cell survival after cytotoxic insult by testing the enzymatic acitivity of the mitochondria. It is based on the reduction of the water-soluble tetrazonium salt to a purple, insoluble formazan derivative by mitochondrial enzyme dehyrogenases. This enzymatic function is only present in living, metabolically active cells. The optical density of the product was quantified by its absorption at 540 nm using a 96-well ELISA plate reader (iEMS Reader MF, Labsystems, USA). MTT, 0.5 mg/ml, was added to each well and incubated for 2 h at 37°C. The medium was then removed and the cells were washed with PBS solution. For cell lysis and dissolution of the formazan crystals formed, 250 μl of isopropanol containing 1% 4 N HCl were added, and the absorption of each residue was determined by using the plate reader at 540 nm. Absorbance of the solution from cells incubated with ALA or its derivatives was divided by the absorption of the solution from the control cell plates t calculate the fraction of surviving cells.

2.6. Concentration and time dependence of PpIX formation

The influence of precursor concentration on the total amount of PpIX formed was measured by permanent incubation of the different cell lines with a given ALA derivative dissolved in PBS at pH 7.4. PpIX was measured 3 and 6 h after drug exposure. Concentration-dependent saturation of PpIX biosynthesis in A549 cell cultures was determined by using different concentrations (0.1–2 mM) of h-ALA. Cells were incubated with a medium containing 5% FCS and fluorescence measurements were carried out every 30 min during 24 h. The influence of the presence of FCS on the PpIX formation was examined by incubation of A549 cells with a 0.8 mM solution of h-ALA containing no, 1%, and 5% FCS, respectively. In order to correct all data for background autofluorescence, in each experiment six wells were incubated without any PpIX precursor.

2.7. Pharmacokinetic studies

PpIX formation in cells incubated with different derivatives of ALA was followed over a period of 5 h. For this purpose, the cells were incubated with the corresponding PpIX precursor at its optimal concentration (as determined according to the above-mentioned procedure). Measurements of fluorescence intensity were taken every 15 min.

2.8. pH Dependence of PpIX formation

The impact of initial extracellular pH was determined using solutions of ALA, h-ALA, and ch-ALA in sterile, isotonic NaCl (aq., 0.9%). The initial pH values, ranging between 5.5 and 8.5, were adjusted with 1 N NaOH for ALA and 0.1 N HCl for ALA derivatives. ALA and its derivatives were applied using concentrations lower than the optimal concentration, typically $c_{opt}/2$. Fluorescence intensity was measured immediately after incubation and again after 3 h. Cell viability was tested as described in Section 2.5.

3. Results and discussion

3.1. Physicochemical properties

The lipophilicity of ALA and its derivatives was assessed by measuring the apparent partition coefficient (P) of the compounds between octanol and a PBS solution of pH 7.4. Table 2 summarizes the obtained log P values. The results plotted in Fig. 2 show that it is possible to vary the lipophilicity of ALA by more than three orders of magnitude when using ALA esters. The log P values of ALA and m-ALA are negative, representing the hydrophilic feature of these substances. Relative to ALA and m-ALA, all other esters are more lipophilic with positive log P values. Both ALA and its esters are highly protonated at pH 7.4 due to the 5-amino group. Therefore the apparent partition coefficient may be

Table 2
Log P and pK_a values for ALA and its derivatives. P is the partition coefficient between octanol and aqueous buffer solution (pH 7.4, 21°C)

Compound	log P	pK_{a1}	pK_{a2}
ALA	−1.51692	4.1±0.1	8.7±0.2
m-ALA	−0.94233		8.4±0.3
e-ALA	0.84113		8.4±0.2
b-ALA	1.42315		8.3±0.1
h-ALA	1.83883		8.3±0.3
o-ALA [a]	2.6199		
ch-ALA	1.49392		8.3±0.2

[a] pK_{a2} not measurable because of precipitation.

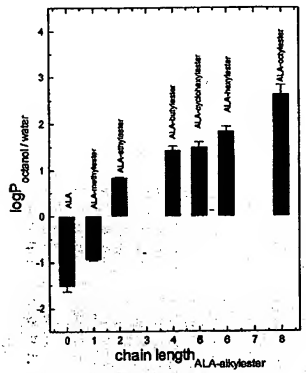

Fig. 2. Derived log P values for ALA and its derivatives.

dependent on the relative amount of uncharged molecules in the aqueous phase. The percentage of molecules with an unprotonated amino group can be calculated using the measured pK_a values (see Table 2). For the different derivatives, this percentage varies between 6 and 12%. The pK_{a1} and pK_{a2} values for ALA given in Table 2 are in good agreement with the results of Novo et al. [24]. The deprotonation of the amino group in o-ALA results in a reversible precipitation of the product in aqueous solution at pH ~ 8 and at a concentration around 20 mM. Furthermore, cleavage of some esters occurs in basic (pH > 9) solutions (data not shown) [25].

Systematic studies of Bridges et al. [26] with a series of homologue carbamates have shown a relatively constant absorption rate for compounds with log P values ranging between 0.8 and 2.8. However, carbamates with log P values less than 0.8 have shown reduced bladder-wall absorption. This suggests a higher tissue uptake for ALA esters containing two or more carbon atoms in their ester function.

Besides higher solubility of compounds with higher lipophilicity in creams and ointments, the data presented in Table 2 have additional impact for the use of ALA esters in dermatology. One of the principal functions of the skin, in particular the stratum corneum (SC), is to avoid the absorption of compounds that come in contact with the skin's surface. Using an approximation based on the analysis of 90 compounds [18], one can estimate the steady-state permeability coefficient K_p of ALA derivatives. It can be calculated that b-ALA will be transported about 50 times more efficiently into the skin than ALA while, using m-ALA, this uptake rate will only be doubled. However, the magnitude of P is important in terms of drug bioavailability. Substances that are too lipophilic may be accumulated in the SC, which consists primarily of free fatty acids, cholesterol, and ceramides. In order to obtain maximal flux across the entire skin, a balanced partition coefficient and good water and lipid solubility are required. Furthermore, the intrinsic solubility may be modified by co-diffusing formulation components.

Additionally, one should bear in mind that facilitated drug uptake does not automatically mean higher PpIX formation. Esters of ALA must be cleaved by esterases before entering the ordinary biosynthetic pathway of heme. These enzymes may have a more or less marked affinity to certain ester functions [21].

3.2. Influence of concentration on PpIX accumulation

The amount of porphyrin biosynthesis resulting from incubation of cells with ALA or its derivatives was determined by measuring the intensity of PpIX fluorescence.

All cell lines displayed the capability to produce PpIX when exposed to ALA or a prodrug given in Table 1. Since FCS has been shown to provoke efflux of PpIX in several cell lines [27], we incubated A549 cells with h-ALA for 5 h with and without FCS. Under our experimental conditions no influence of FCS on the total amount of PpIX generated has been found (see Table 3). The effect of concentration was assessed using ALA or ALA prodrug concentrations varying over two orders of magnitude. As shown in Fig. 3, there was a dose-dependent PpIX accumulation for each cell line and for each PpIX precursor used. The shape of the dose–response curves was similar in each case. While PpIX generation is positively correlated up to an optimal prodrug concentration (c_{opt}) where the highest PpIX fluorescence levels occurred, PpIX generation decreases when this threshold concentration is exceeded. The absolute value of the optimal concentrati n varies with the type of prodrug and cell line. Except for the

Table 3
Influence of FCS on the PpIX formation in A549 cells after 5 h of incubation with 0.8 mM of h-ALA

FCS (%)	PpIX fluorescence [a.u.]
0	680±150
1	670±170
5	680±140

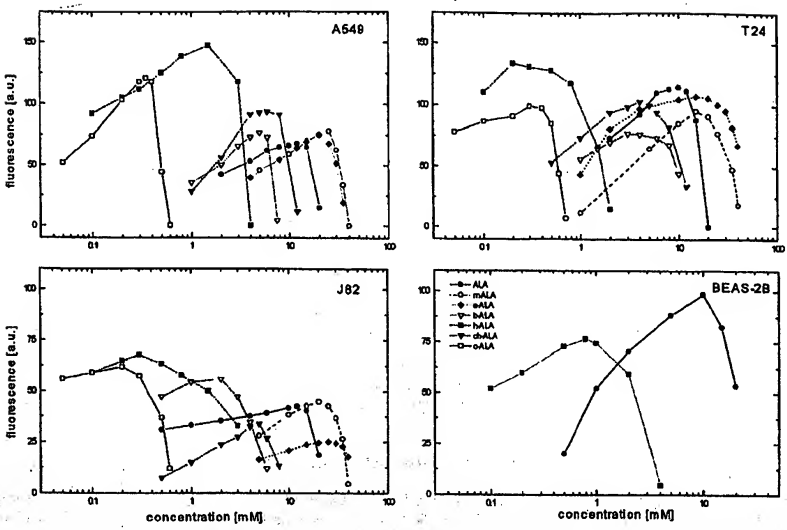

Fig. 3. Concentration dependence of PpIX accumulation for four different human cell lines after 3 h of incubation with ALA (●), m-ALA (○), e-ALA (♦), b-ALA (△), h-ALA (■), ch-ALA (▼), and o-ALA (□) (standard deviations (SD) have been omitted for the sake of clarity, see Fig. 5 for exemplary SD).

BEAS-2B cell line, incubation with h-ALA resulted in the highest fluorescence levels.

In general, the c_{opt} values for m-ALA and e-ALA were higher than for ALA. ALA esters with alkyl groups consisting of four carbon atoms or more (b-ALA, h-ALA, and o-ALA) showed their optimal PpIX formation at significantly lower c_{opt} values (Fig. 4). Furthermore, the bandwidth of the dose–response curves for these esters was always smaller than for ALA, m-ALA, or e-ALA (Fig. 3), indicating that the choice of the optimal concentration is crucial in order to guarantee an optimal PpIX generation.

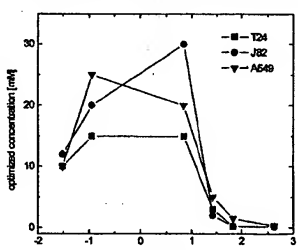

Fig. 4. Concentration of ALA and its derivatives needed to induce maximal PpIX accumulation after 3 h of incubation on A549 (▼), T24 (■), and J82 ( ) cell lines. See also Table 3.

Similar fluorescence intensity–concentration profiles were measured after 300 min of incubation (data not shown). Both the value of c_{opt} and the bandwidth of the dose–response curves remained unchanged. The long-term influence of permanent drug exposure on PpIX biosynthesis was tested by incubation of A549 cells with h-ALA, which has shown the most promising results under our conditions with respect to its dose–response behavior (Fig. 5). For concentrations smaller than c_{opt}, PpIX formation increases in a moderate sigmoidal way with incubation time (Fig. 5(a)). Under optimal conditions, continuously increasing PpIX accumulation can be observed for 24 h. Depending on the concentration, the linear part of these curves ends between 4 and 15 h and proceeds into a moderate plateau. For small concentrations the height of this plateau depends linearly on the concentration of h-ALA, whereas higher concentrations show a saturation of the PpIX biosynthesis (Fig. 5(b)). This might indicate a saturation of the enzymatic functions. However, higher concentrations than c_{opt} end with less PpIX formed, although no reduced cell viability has been determined under these conditions (see below).

No direct correlation between log P and c_{opt} or the amount of PpIX produced can be noted from the data in the present work (Fig. 4). Excluding ch-ALA, how ver, a decrease of

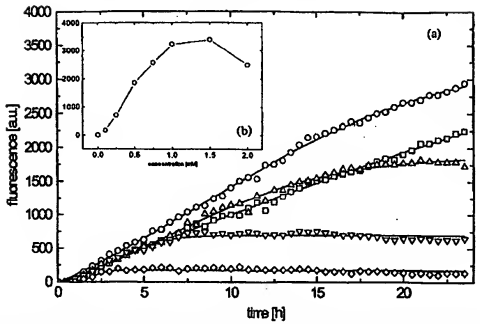

Fig. 5. (a) Pharmacokinetics of PpIX synthesis in A549 cells treated with different concentrations of h-ALA during 24 h of permanent drug exposure. Concentrations: 2 mM (□), 1.5 mM (○), 0.5 mM (△), 0.25 mM (▽), and 0.1 mM (◇). (b) PpIX formation after 24 h as a function of concentration of h-ALA solutions (for description see Section 2).

c_{opt} with increasing chain length for lipophilic ALA derivatives (starting from e-ALA) can be established.

While long-chained derivatives ($C \geq 4$) showed lower c_{opt} values than ALA, m-ALA as well as e-ALA seemed to be less efficient, although more lipophilic than ALA. The combination of two distinct processes may explain this behavior. While lipophilicity defines the transport of a drug across cell membranes, ALA esters must be cleaved by nonspecific esterases prior to entering the biosynthetic pathway of heme. Kloek et al. [21] have shown with cell lysates that enzymatic hydrolysis is faster for long-chained esters than for short-chained esters. This information might have an impact for further synthesis of derivatives of ALA. Such new prodrugs should have a similar lipophilicity to h-ALA or o-ALA and the enzymatic cleavage of the ester function should also be optimized. Derivatives of ALA can be adapted to specific esterases of tumor cells for further improvement of the selectivity of ALA-induced PpIX.

Similar dose-dependence characteristics have also been observed by other groups with ALA [28–32]. These groups found either a saturation of PpIX or a slight decrease of the resulting PpIX fluorescence with increasing ALA concentration. Gaullier et al. [22] observed an optimal concentration on different human and animal cell lines for long-chained ALA esters in the same order of magnitude as presented in this work. The more than twofold increase of the PpIX formation rate with ALA esters as compared with ALA is in good agreement with the results we recently obtained from measurements on an organ culture model [19]. Recently, Kloek et al. [21] compared the performance of different ALA derivatives on human lymphoma cell lines. They found that ALA pentyl ester induced the highest PpIX levels in intact cells, while h-ALA and b-ALA have shown similar fluorescence intensities after 6 h of incubation. However, in the course of their experiments, incubation was performed using equimolar concentrations for all derivatives. Hence, it might be possible that for long-chained alkyl esters the concentration was too high to produce large amounts of PpIX.

The relative rate of PpIX generation increases with increasing lipophilicity of the corresponding ALA ester from m-ALA to h-ALA, whereas comparable rates of h-ALA and o-ALA suggest a saturation of some enzymatic functions in the biosynthetic pathway within these time ranges. Taking into account c_{opt}, which was 10–100 times lower for long-chained ALA esters than for ALA, it can be concluded that, using such compounds, the PpIX formation efficiency was enhanced by almost two orders of magnitude by simple chemical derivatization.

Since ALA is known to induce cytotoxic effects in cell culture [33–35], the PpIX accumulation observed was evaluated with respect to the cell viability after incubation with different concentrations of each PpIX precursor. It can be seen from Fig. 6 that the reduction of PpIX formation after incubation with drug doses higher than c_{opt} coincides with a reduced cell survival. This correlation was observed for all cell lines and prodrugs. Incubation with lower doses of ALA or its derivatives did not affect cell viability, as confirmed by the MTT test. From these experiments, it is obvious that only well-defined drug doses will improve the PpIX formation in clinical applications when using ALA esters instead of ALA.

3.3. pH Dependence of PpIX formation

It has been found that tumor tissues are generally more acidic than surrounding normal tissues. This is probably due to an overproduction of lactic acid and hydrolysis of adenosine triphosphate (ATP). Since this microenvironmental factor may influence PpIX generation, we incubated three cell lines with ALA, ch-ALA, and h-ALA solutions adjusted to pH values in the range between 5.5 and 8.5. In order to prevent

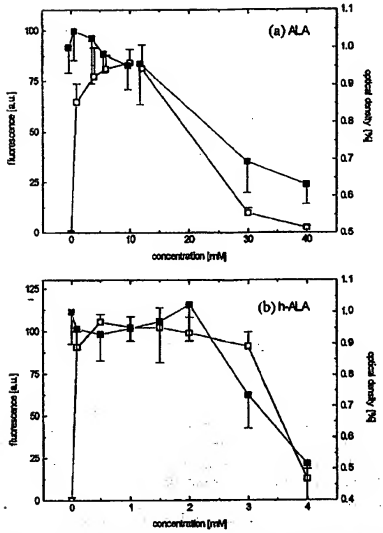

Fig. 6. Correlation of PpIX fluorescence intensity (□) and mitochondrial activity test by means of an MTT assay (■) in A549 cell for (a) ALA and (b) h-ALA (for description see Section 2).

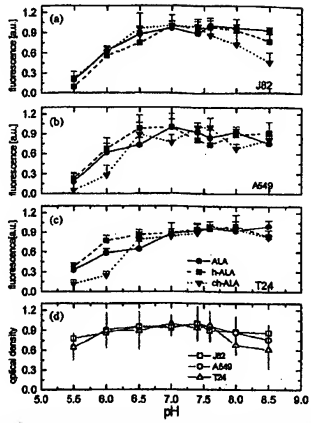

Fig. 7. PpIX formation as a function of initial pH values in (a) J82, (b) A549 and (c) T24 cell lines after 120 min of incubation with 0.1 mM h-ALA (■), 3.2 mM ch-ALA (▼), and 5 mM ALA (●) solutions (fluorescence values are normalized to the maximum of fluorescence). (d) Mitochondrial activity test as a function of initial pH values.

saturation or cytotoxic effects provoked by the drug itself, concentrations lower than c_{opt} ($c_{opt}/2$) were chosen. The values plotted in Fig. 7 indicate that optimal PpIX formation occurs at physiological pH values of around 7.5 ± 0.5. The total PpIX production approximately tripled at pH 7.4 compared with the production induced at pH 5.5. Due to proton release to the nonbuffered medium, the initial pH values decreased during incubation. While under alkaline conditions this effect was more marked ($\Delta pH \sim 0.3/h$ at 8.5), the pH values under acidic conditions remained nearly unchanged ($(\Delta pH \sim 0.01/h$ at pH 6.5)). Generally, PpIX production was more drastically reduced under acidic than under alkaline conditions, extending previously published results using ALA as a PpIX precursor [28,36,37]. While the decrease of PpIX formation at higher pH values can be attributed to a reduction of cell viability, the decrease under acidic conditions can be attributed to either a pH-dependent drug uptake or a reduced enzymatic activity in the biosynthesis of heme. As has been shown, ALA uptake is regulated by a pH-dependent ion pump that is more active at pH 5.0 [38]. Hence, PpIX production would be expected to increase under acidic conditions. However, it is known that intracellular pH is also downregulated when extracellular pH falls below 6.5 [39]. This might inhibit the activity of enzymes involved in the biosynthetic pathway of heme, which have optimal activity between pH 7 and 7.5 [37]. A further indication for this pH-dependent intracellular process is given by the use of ALA esters, since a major part of these amphiphilic compounds will be taken up actively, as demonstrated by inhibitory tests [40].

From these experiments, it can be concluded that, for diagnostic as well as for therapeutic reasons, ALA formulations adjusted to physiological pH values should be applied. However, the instability of ALA implies the administration of ALA solution adjusted to lower pH values for the photodetection of early human bladder cancer in urology [6–9,24]. Since these are physiological pH values for urothelial cells, the uptake of ALA may not be affected by solutions buffered to a pH of 5. In contrast, the production of PpIX under these conditions may be strongly dependent on this parameter. Novo and colleagues [24] have attributed the chemical instability of ALA to an irreversible dimerization of two parent molecules followed by an oxidation of the resulting dihydropyrazine derivative. Generally, the velocity of such bimolecular reactions is proportional to the product of the concentrations of the two involved reactants. Hence, under this assumption the drastic reduction of the concentration by a factor of about 20 [22] that is used with ALA esters enhances the stability of the corresponding solution by a factor of 400. This increase of stability opens the possibility for a further increase of PpIX formation after topical application of ALA derivatives by a simple adjustment of the pharmaceutical formulation to physiological pH values.

4. Conclusions

In summary, this study shows that using esters of ALA instead of ALA indicates a promising route to improve many clinical applications of PpIX-mediated PDT and fluorescence photodetection. Th faster intracellular build-up of PpIX and the drastically reduced concentration relative to ALA enables treatments with significantly lower doses and shorter application times. Therefore, a significant decrease in costs should be associated with the use of such esters. Faster production of PpIX and hence shorter instillation times may play an important role for commercialization of this technique. Moreover, the enhancement of lipophilicity, which has been achieved by esterification, will result in deeper penetration of the drug into targeted lesions after topical application and possibly also in a more homogeneous distribution of the resulting photosensitizer. Therefore, more efficient PDT mediated by such prodrugs will be possible. No direct relationship between lipophilicity and total PpIX build-up has been found, indicating that two different processes, uptake and ester cleavage, are necessary for efficient PpIX formation. Moreover, long-chained esters should always be applied with lower doses than ALA. In most cell lines h-ALA has shown the most efficient PpIX formation.

A further enhancement of PpIX formation can be obtained by an adjustment of applied ALA and ALA prodrug formulations to physiological pH values.

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